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Washington, D.C. 20231

Elan Pharmaceutical Research Corporation
1300 Gould Drive
Gainesville, GA 30504

November 19, 1999

Dear Sir:

Transmitted herewith for filing are the specification and claims of the patent application of:

Inventor(s): **O'Mahony, Daniel Joseph**

Title of Invention: **"Retro-inversion peptides that target GIT receptors and related methods"**

Also enclosed are:

X	2 Sheets of	[X] FORMAL DRAWINGS	[ ] INFORMAL DRAWINGS
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X	A POWER OF ATTORNEY		
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Respectfully submitted,

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TO ALL WHOM IT MAY CONCERN:

Be it known that I, Daniel J. O'Mahony, a citizen of Ireland, residing at 75 Avoca Park, Avoca Avenue, Blackrock County, Dublin, Ireland, have invented new and useful improvements in

Retro-inversion peptides that target GIT transport receptors and related methods

for which the following is a specification.

Retro-inversion peptides that target GIT transport receptors and related methods

FIELD OF THE INVENTION

The present invention relates generally to peptides that are capable of targeting or specifically binding to gastro-intestinal tract (GIT) transport receptors. In particular, this invention relates to retro-inverted forms of peptide sequences and motifs, as well as derivatives thereof, which enhance drug delivery and transport through tissue, such as epithelial cells lining the lumenal side of the GIT. Production of peptides and antibodies is also provided. The invention further relates to pharmaceutical compositions, formulations and related methods.

BACKGROUND OF THE INVENTION

Proteases cleave peptide bonds between adjacent L-amino acids, rendering these peptides susceptible to degradation in the GIT. Artificial proteins or peptides composed of D-amino acids are largely resistant to proteolytic breakdown. However, when D-amino acids are substituted for all L-amino acids in a peptide/protein, the corresponding D-peptide/protein is a mirror image of the original peptide/protein and is likely to have modified or lost biological activity because of this change in conformation. Retro-inverted peptides are peptides having all D-amino acids but are synthesized in the reverse order or sequence compared to the original L-peptide/protein. The carboxy terminus of the original peptide/protein becomes the amino terminus (and vice versa) of the retro-inverted peptide/protein and the resulting side chain surface of the retro-inverted peptide/protein is similar to the original L-peptide/protein. The net result of combining D-enantiomers and reverse synthesis is that the positions of carbonyl and amine groups in each amide bond are exchanged while the position of side-chain groups is preserved (Brady, L. and Dodson, G., *Nature*, 368L:692-693 (1994); Jameson et al.,

Nature, 368;744-746 (1994)). This alteration in the protein backbone is self compensating in that hydrogen-bond donors become hydrogen-bond acceptors (amide carbonyl groups) and vice-versa. When the position of the side-chains relative to the backbone are unchanged the modified surface of the retro-inverted peptide/protein is largely unaltered compared to the original L-peptide/protein.

Previously, as disclosed and claimed in WO 98/51325, which is hereby incorporated by reference in its entirety, we have identified random peptides and their fragments, motifs, derivatives, analogs or peptidomimetics thereof which are capable of specific binding to GIT transport receptors such as the D2H, hSI, HPT1 and hPEPT1 receptors (hereinafter "GIT targeting agents"). These GIT targeting agents are capable of facilitating transport of an active agent through a human or animal gastro-intestinal tissue and have use, for example, in facilitating transport of active agents from the lumenal side of the GIT into the systemic blood system and/or in targeting active agents to the GIT. Thus, for example, by binding (covalently or noncovalently) the GIT targeting agent to an orally administered active agent, the active agent can be targeted to specific receptor sites or transport pathways which are known to operate in the human gastrointestinal tract, thus facilitating its absorption into the systemic system. Preferably, the active agent is a drug or a drug-containing nano- or microparticle.

SUMMARY OF THE INVENTION

Surprisingly, we have found that retro-inverted forms of the GIT targeting agents target specific receptor sites *in vivo* and/or promote uptake of active agents and/or enhance active agent delivery across the GIT into the systemic circulation. By using retro-inversion D-peptide synthesis, we have discovered retro-inverted D-peptides of the GIT targeting agents that retain the same function of the GIT targeting agents but have enhanced stability to proteases in the human or animal GIT. These retro-inverted peptides and

compositions containing these retro-inverted peptides can be used to deliver an active agent, such as a drug or a drug-containing nano- or microparticle for treatment of a condition in a subject in need of the drug, *in vivo* by any of the uses or methods disclosed in the above-referenced WO 98/51325. Additionally, the invention provides antibodies which are capable of immunospecifically binding the retro-inverted peptides of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the systemic blood glucose levels following intestinal administration of control (PBS); ZElan 021 coated insulin-containing particles, ZElan 018 coated insulin-containing particles, ZElan091 coated insulin-containing particles, ZElan129 coated insulin-containing particles and ZElan 144 coated insulin-containing particles according to this invention (300iu insulin loading); and

Figure 2 shows the systemic insulin levels following intestinal administration of control ZElan 021 coated insulin-containing particles, ZElan 018 coated insulin-containing particles, ZElan091 coated insulin-containing particles, ZElan129 coated insulin-containing particles and ZElan144 coated insulin-containing particles according to this invention (300iu insulin loading).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to retro-inverted peptides (also referred to herein as "targeting retro-inverted peptides" or "targeting retro-inversion peptides") that target specific receptor sites *in vivo* and/or promote uptake of active agents and/or enhance active agent delivery across the GIT into the systemic, portal or hepatic circulation. In particular, these retro-inverted peptides specifically bind to one or more of the human gastro-intestinal tract receptors HPT1, HPEPT1, D2H or hSI or their equivalents in other mammals and have general utility in targeting active agents to selected sites and/or selected tissues in the body in which the receptors are expressed. These

peptides are synthesized from D-amino acids and have a reverse sequence order of the GIT targeting agents disclosed and claimed in the above-referenced WO 98/51325. The present invention also relates to derivatives (including but not limited to fragments) of these retro-inverted peptides, which derivitives are functionally similar to the retro-inverted peptides (that is, capable of displaying one or more known functional activities of the retro-inverted peptides). These functional activities include but are not limited to the ability to bind or to compete with binding to the gastro-intestinal tract receptors HPT1, HPEPT1, D2H or hSI or the ability to be bound by an antibdy directed against the retro-inverted peptide. Derivatives can be tested for the desired activity by procedures known in the art, including binding to a receptor domain or to Caco-2 cells, *in vitro,* or to intestinal tissue, *in vitro* or *in vivo*.

Derivatives can be made by altering the retro-inverted peptides sequences by substitutions, additions or deletions that provide for functionally equivalent activity. Derivatives include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of the retro-inverted peptide including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequences resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Included within the scope of the invention are retro-inverted peptides or derivatives which are modified. e.g., by glycosoylation, acetylation,

phosphorylation, amidation, derivitization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques. In a specific embodiment, the amino- and/or carboxy-termini are modified. Furthermore, is desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the retro-inverted peptides sequence. Non-classical amino acids include but are not limited to α-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ-Abu, ε-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, Cα-methyl amino acids, Nα-methyl amino acids, and amino acid analogs in general.

The present invention also relates to therapeutic and diagnostic methods and compositions containing the targeting retro-inverted peptides.

The invention provides compositions comprising the targeting retro-inversion peptides of the invention bound to a material comprising an active agent. Such compositions have use in targeting the active agent to the GIT and/or in facilitating transfer through the lumen of the GIT into the systemic circulation in a human or animal subject. The retro-inverted D-peptides also have general utility in targeting active agents to selected sites/selected tissues in a human or animal subject in which the receptors or other related receptors are expressed. For instance, where the retro-inverted D-peptides bind to other receptors, such as related receptors, splice variants of the receptors or related receptors which exist as a superfamily, or where the peptides bind through non-specific interactions, such as non-specific ion-pairings, hydrogen bonding or hydrophobic pairings, they can be used to deliver drugs to tissues or cell types in mammals or humans that express these receptors. Additionally, when the active agent is an imaging agent,

such compositions can be administered *in vivo* to image selected sites/selected tissues, such as the GIT (or particular transport receptors thereof). Other active agents include but are not limited to: any drug or antigen or any drug- or antigen-loaded or drug- or antigen-encapsulated nanoparticle, microparticle, liposome, or micellar formulation capable of eliciting a biological response in a human or animal. Examples of drug- or antigen-loaded or drug- or antigen-encapsulated formulations include those in which the active agent is encapsulated or loaded into nano- or microparticles, such as biodegradable nano- or microparticles, and which have the targeting retro-inversion peptide adsorbed, coated or covalently bound, such as directly linked or linked via a linking moiety, onto the surface of the nano- or microparticle. Additionally, the targeting retro-inverted peptide can form the nano- or microparticle itself or the targeting retro-inverted peptide can be covalently attached to the polymer or polymers used in the production of the biodegradable nano- or microparticles or drug-loaded or drug-encapsulated nano- or microparticles or the peptide can be directly conjugated to the active agent. Such conjugation to active agents include proteins in which the retro-inverted peptide is conjugated directly to the protein or peptide active agent of interest. Additionally, the retro-inverted peptides of this invention can be attached to the building blocks or subunits or polymer monomers used in the synthesis of the base polymers.

In a preferred embodiment, the invention provides for treatment of various diseases and disorders by administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include but are not limited to: targeting retro-inversion peptide bound to an active agent of value in the treatment or prevention of a disease or disorder (preferably a mammalian, most preferably human, disease or disorder). The active agent is preferably a drug.

Any drug known in the art may be used, depending upon the disease or disorder to be treated or prevented, and the type of subject to which it is to be administered. As used herein, the term "drug" includes, without limitation,

any pharmaceutically active agent. Representative drugs include, but are not limited to, peptides or proteins, hormones, analgesics, anti-migraine agents, anti-coagulant agents, anti-emetic agents, cardiovascular agents, anti-hypertensive agents, narcotic antagonists, chelating agents, anti-anginal agents, chemotherapy agents, sedatives, anti-neoplastics, prostaglandins, and antidiuretic agents. Typical drugs include peptides, proteins or hormones such as insulin, calcitonin, calcitonin gene regulating protein, atrial natriuretic protein, colony stimulating factor, betaseron, erythropoietin (EPO), interferons such as α, β or γ interferon, somatropin, somatotropin, somatostatin, insulin-like growth factor (somatomedins), luteinizing hormone releasing hormone (LHRH), tissue plasminogen activator (TPA), growth hormone releasing hormone (GHRH), oxytocin, estradiol, growth hormones, leuprolide acetate, factor VIII, interleukins such as interleukin-2, and analogs thereof; analgesics such as fentanyl, sufentanil, butorphanol, buprenorphine, levorphanol, morphine, hydromorphone, hydocodone, oxymorphone, methadone, lidocaine, bupivacaine, diclofenac, naproxen, paverin, and analogs thereof; anti-migraine agents such as heparin, hirudin, and analogs thereof; anti-coagulant agents such as scopolamine, ondansetron, domperidone, etoclopramide, and analogs thereof; cardiovascular agents, anti-hypertensive agents and vasodilators such as diltiazem, clonidine, nifedipine, verapamil, isosorbide-5-mononitrate, organic nitrates, agents used in treatment of heart disorders and analogs thereof; sedatives such as benzodiazeines, phenothiozines and analogs thereof; narcotic antagonists such as naltrexone, naloxone and analogs thereof; chelating agents such as deferoxamine and analogs thereof; anti-diuretic agents such as desmopressin, vasopressin and analogs thereof; anti-anginal agents such as nitroglycerine and analogs thereof; anti-neoplastics such as 5-fluorouracil, bleomycin and analogs thereof; prostaglandins and analogs thereof; and chemotherapy agents such as vincristine and analogs thereof. Representative drugs also include but are not limited to antisense oligonucleotides, genes, gene correcting hybrid oligonucleotides, ribozymes, aptameric oligonucleotides, triple-helix forming oligonucleotides, inhibitors of signal transduction pathways, tyrosine kinase

inhibitors and DNA modifying agents. Drugs that can be used also include, without limitation, systems containing gene therapeutics, including non-viral systems for therapeutic gene delivery and viral vector systems for therapeutic genes which are modified with a retro-inversion peptide post virus purification.

In a preferred embodiment, a Therapeutic is therapeutically or prophylactically administered to a human patient.

Additional descriptions and sources of Therapeutics that can be used according to the invention are found in various Sections herein.

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably a human.

As will be clear, any disease or disorder of interest amenable to therapy or prophylaxis by providing a drug *in vivo* systemically or by targeting a drug (by linkage to a targeting retro-inversion peptide of the present invention) *in vivo* to the GIT or other selected sites, selected tissues or cell types which contain the receptor or other receptors, such as related receptors, splice variants of the receptors, related receptors which exist as a superfamily or to which the retro-inverted peptide interacts through non-specific interaction, such as non-specific ion-pairings or hydrogen bondings or hydrophobic pairings (using any route of administration) can be treated or prevented by administration of a Therapeutic of the invention. Such diseases may include but are not limited to hypertension, diabetes, osteoporosis, hemophilia, anemia, cancer, migraine, and angina pectoris, to name but a few.

Any route of administration known in the art may be used, including but not limited to oral, nasal, topical, intravenous, intraperitoneal, intradermal,

mucosal, intrathecal, intramuscular, etc. Preferably, administration is oral; in such an embodiment the targeting retro-inverted peptide according to this invention acts advantageously to facilitate transport of the therapeutic active agent through the lumen of the GIT into the portal, hepatic or systemic circulation.

The present invention also provides therapeutic compositions or formulations. In a specific embodiment of the invention, a targeting retro-inversion peptide is associated with a therapeutically or prophylactically active agent, preferably a drug or drug-containing nano- or microparticle. More preferably, the active agent is a drug encapsulating or drug loaded nano- or microparticle, such as a biodegradable nano- or microparticle, in which the peptide is physically adsorbed or coated or covalently bonded, such as directly linked or linked via a linking moiety, onto the surface of the nano- or microparticle. Alternatively, the peptide can form the nano- or microparticle itself or can be directly conjugated to the active agent. Preferably the particles range in size from 10nm and 500 μm, more preferably 50 to 800 nm, most preferably 200-600 nm.

Thus, in a specific embodiment, a targeting retro-inversion peptide is bound to a slow-release (controlled release) device containing a drug. In a specific embodiment, polymeric materials can be used (*see* Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); *see also* Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)).

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a

regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, sorbitol, trehelose and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient.

The active agent of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as

those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

According to this invention, a targeting retro-inverted peptide may also be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and a Fab expression library.

These antibodies can be used in methods relating to the localization and activity of the targeting retro-inversion peptide sequences of the invention, *e.g.*, for imaging these peptides after *in vivo* administration (*e.g.*, to monitor treatment efficacy), measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc. For instance, antibodies or antibody fragments specific to a domain of a targeting retro-inversion peptide, such as a dansyl group or some other epitope introduced into the peptide, can be used to 1) identify the presence of the peptide on a nanoparticle or other substrate; 2) quantify the amount of peptide on the nanoparticle; 3) measure the level of the peptide in appropriate physiological samples; 4) perform immunohistology on tissue samples; 5) image the peptide after *in vivo* administration; 6) purify the peptide from a mixture using an immunoaffinity column, 7) bind or fix the peptide to the surface of nanoparticle or 8) when a tag is also added to either an active-agent containing particle or the active agent itself, track the fate of both the particle/active agent and the

targeting retro-inversion peptide so as to determine if and/or where they become separated. Use 7 above envisions attaching the antibody (or fragment of the antibody) to the surface of drug-loaded nanoparticles or other substrates and then incubating this conjugate with the peptide. This procedure results in binding of the peptide in a certain fixed orientation, resulting in a particle that contains the peptide bound to the antibody in such a way that the peptide is fully active. Additionally, antibodies or antibody fragments specific to a domain of a targeting retro-inverted peptide 9) can be used in confocal microscopy imaging techniques or other imaging techniques in order to demonstrate or confirm or identify the location or localization of the peptide on the surface of a nano- or microparticle, 10) can be used in confocal microscopy imaging techniques or other imaging techniques in order to demonstrate or confirm or identify the location or localization of the peptide on the surface of a nanoparticle or microparticle which has also been loaded with a fluorescent agent, 11) in the case of nanoparticles or microparticles coated with the peptide which have been sliced into two halves by a microtone or other suitable techniques, the antibody can be used in suitable quantitative techniques such as confocal microscopy imaging techniques or other quantitative imaging techniques in order to identify or quantitate the relative distribution of the peptide between the surface of the nanoparticle or microparticle and the sub-surface interior matrix of the nanoparticles or microparticles, 12) can be used in confocal microscopy imaging techniques or other imaging techniques in order to demonstrate or confirm or identify the location of a peptide on the surface of a nanoparticle or microparticle which has been loaded with a fluorescent agent such as TRME or fluorascene, 13) can be used to identify which epitope or domain of the peptide is responsible for identification by the antibody; peptide derivatives such as cyclic forms or derivatives containing intra-chain disulphide bonds or other intra-chain bonds can also be used in mapping studies in order to identify which domain or epitope of the peptide is responsible for recognition by the antibody; 14) in the case of peptide derivatives in which the epitope or domain responsible for binding to a target receptor is flanked by di-sulphide bond or other intra-chain

bonds and in which this domain is also responsible for binding to the antibody, the antibody can be used to determine if that epitope or domain is exposed or available for binding to the antibody when the peptide or derivative is coated onto the surface of a nanoparticle, microparticle or other substance, 15) can be used where the epitope or domain on the peptide which binds to the target receptors in the human gastro-intestinal tract or the target receptors on model epithelial cells such as Caco-2 cells or polarised Caco-2 cells and where this epitope or domain on the peptide is also responsible for binding by the antibody, the antibody can be used in competition studies to compete for the binding of the peptide to its target receptor sites and 16) where the epitope or domain on the peptide which binds to the target receptors in the human gastro-intestinal or the target receptors on model epithelial cells such as Caco-2 cells or polarised Caco-2 cells and where this epitope or domain on the peptide is also responsible for binding by the antibody, the antibody can be used in competition studies in which nanoparticles or microparticles are coated with the peptide and are used in cell binding studies and / or in receptor binding studies.

Abtides (or Antigen binding peptides) specific to a domain of targeting retro-inverted peptide, such as a dansyl group or some other epitope introduced into the peptide, can be used for the same purposes identified above for antibodies.

The retro-inverted peptides of this invention may be prepared by methods that are known in the art. For example, in brief, solid phase peptide synthesis consists of coupling the carboxyl group of the C-terminal amino acid to a resin and successively adding N-alpha protected amino acids. The protecting groups may be any known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The coupling of amino acids to appropriate resins is described by Rivier et al., U.S. Patent No. 4,244,946. Such solid phase syntheses have been described, for example, by Merrifield, 1964, J. Am. Chem. Soc. 85:2149; Vale et al., 1981, Science 213:1394-1397; Marki et

al., 1981, J. Am. Chem. Soc. 103:3178 and in U.S. Patent Nos. 4,305,872 and 4,316,891. In a preferred aspect, an automated peptide synthesizer is employed.

By way of example but not limitation, peptides can be synthesized on an Applied Biosystems Inc. ("ABI") model 431A automated peptide synthesizer using the "Fastmoc" synthesis protocol supplied by ABI, which uses 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate ("HBTU") (R. Knorr et al., 1989, Tet. Lett., 30:1927) as coupling agent. Syntheses can be carried out on 0.25 mmol of commercially available 4-(2',4'-dimethoxyphenyl-(9-fluorenyl-ethoxycarbonyl)-aminomethyl)-phenoxy polystyrene resin ("Rink resin" from Advanced ChemTech) (H. Rink, 1987, Tet. Lett. 28:3787). Fmoc amino acids (1 mmol) are coupled according to the Fastmoc protocol. The following side chain protected Fmoc amino acid derivatives are used: FmocArg(Pmc)OH; FmocAsn(Mbh)OH; FmocAsp(^{t}Bu)OH; FmocCys(Acm)OH; FmocGlu(^{t}Bu)OH; FmocGln(Mbh)OH; FmocHis(Tr)OH; FmocLys(Boc)OH; FmocSer(^{t}Bu)OH; FmocThr(^{t}Bu)OH; FmocTyr(^{t}Bu)OH. [Abbreviations: Acm, acetamidomethyl; Boc, tert-butoxycarbonyl; ^{t}Bu, tert-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; Mbh, 4,4'-dimethoxybenzhydryl; Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tr, trityl].

Synthesis is carried out using N-methylpyrrolidone (NMP) as solvent, with HBTU dissolved in N,N-dimethylformamide (DMF). Deprotection of the Fmoc group is effected using approximately 20% piperidine in NMP. At the end of each synthesis the amount of peptide present is assayed by ultraviolet spectroscopy. A sample of dry peptide resin (about 3-10 mg) is weighed, then 20% piperidine in DMA (10 ml) is added. After 30 min sonication, the UV (ultraviolet) absorbance of the dibenzofulvene-piperidine adduct (formed by cleavage of the N-terminal Fmoc group) is recorded at 301 nm. Peptide substitution (in mmol g^{-1}) can be calculated according to the equation:

$$\text{substitution} = \frac{A \times v}{7800 \times w} \times 1000$$

where A is the absorbance at 301 nm, v is the volume of 20% piperidine in DMA (in ml), 7800 is the extinction coefficient (in $mol^{-1}dm^3cm^{-1}$) of the dibenzofulvene-piperidine adduct, and w is the weight of the peptide-resin sample (in mg).

Finally, the N-terminal Fmoc group is cleaved using 20% piperidine in DMA, then acetylated using acetic anhydride and pyridine in DMA. The peptide resin is thoroughly washed with DMA, CH_2Cl_2 and finally diethyl ether.

By way of example but not limitation, cleavage and deprotection can be carried out as follows: The air-dried peptide resin is treated with ethylmethyl-sulfide (EtSMe), ethanedithiol (EDT), and thioanisole (PhSMe) for approximately 20 min. prior to addition of 95% aqueous trifluoracetic acid (TFA). A total volume of approximately 50 ml of these reagents are used per gram of peptide-resin. The following ratio is used: TFA:EtSMe:EDT:PhSme (10:0.5:0.5:0.5). The mixture is stirred for 3 h at room temperature under an atmosphere of N_2. The mixture is filtered and the resin washed with TFA (2 x 3 ml). The combined filtrate is evaporated *in vacuo*, and anhydrous diethyl ether added to the yellow/orange residue. The resulting white precipitate is isolated by filtration. See King et al., 1990, Int. J. Peptide Protein Res. 36:255-266 regarding various cleavage methods.

Purification of the synthesized peptides can be carried out by standard methods including chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography, high performance liquid chromatography (HPLC)), centrifugation, differential solubility, or by any other standard technique.

The peptides of the present invention may be linked to other molecules (*e.g.*, a detectable label, a molecule facilitating adsorption to a solid substratum, or a toxin, according to various embodiments of the invention) by methods that are well known in the art. Such methods include the use of homobifunctional and heterobifunctional cross-linking molecules.

The homobifunctional molecules have at least two reactive functional groups, which are the same. The reactive functional groups on a homobifunctional molecule include, for example, aldehyde groups and active ester groups. Homobifunctional molecules having aldehyde groups include, for example, glutaraldehyde and subaraldehyde. The use of glutaraldehyde as a cross-linking agent was disclosed by Poznansky et al., 1984, Science 223:1304-1306.

Homobifunctional molecules having at least two active ester units include esters of dicarboxylic acids and N-hydroxysuccinimide. Some examples of such N-succinimidyl esters include disuccinimidyl suberate and dithio-bis-(succinimidyl propionate), and their soluble bis-sulfonic acid and bis-sulfonate salts such as their sodium and potassium salts. These homobifunctional reagents are available from Pierce, Rockford, Illinois.

The heterobifunctional molecules have at least two different reactive groups. Some examples of heterobifunctional reagents containing reactive disulfide bonds include N-succinimidyl 3-(2-pyridyl-dithio)propionate (Carlsson et al., 1978, Biochem J. 173:723-737), sodium S-4-succinimidyloxycarbonyl-alpha-methylbenzylthiosulfate, and 4-succinimidyloxycarbonyl-alpha-methyl-(2-pyridyldithio)toluene. N-succinimidyl 3-(2-pyridyldithio)propionate is preferred. Some examples of heterobifunctional reagents comprising reactive groups having a double bond that reacts with a thiol group include succinimidyl 4-(N-maleimidomethyl)cyclohexahe-1-carboxylate and succinimidyl m-maleimidobenzoate.

Other heterobifunctional molecules include succinimidyl 3-(maleimido)propionate, sulfosuccinimidyl 4-(p-maleimido-phenyl)butyrate, sulfosuccinimidyl 4-(N-maleimidomethyl-cyclohexane)-1-carboxylate, maleimidobenzoyl-N-hydroxy-succinimide ester. The sodium sulfonate salt of succinimidyl m-maleimidobenzoate is preferred. Many of the above-mentioned heterobifunctional reagents and their sulfonate salts are available from Pierce.

Additional information regarding how to make and use these as well as other polyfunctional reagents may be obtained from the following publications or others available in the art: Carlsson et al., 1978, Biochem. J. 173:723-737; Cumber et al., 1985, Methods in Enzymology 112:207-224; Jue et al., 1978, Biochem 17:5399-5405; Sun et al., 1974, Biochem. 13:2334-2340; Blattler et al., 1985, Biochem. 24:1517-152; Liu et al., 1979, Biochem. 18:690-697; Youle and Neville, 1980, Proc. Natl. Acad. Sci. USA 77:5483-5486; Lerner et al., 1981, Proc. Natl. Acad. Sci. USA 78:3403-3407; Jung and Moroi, 1983, Biochem. Biophys. Acta 761:162; Caulfield et al., 1984, Biochem. 81:7772-7776; Staros, 1982, Biochem. 21:3950-3955; Yoshitake et al., 1979, Eur. J. Biochem. 101:395-399; Yoshitake et al., 1982, J. Biochem. 92:1413-1424; Pilch and Czech, 1979, J. Biol. Chem. 254:3375-3381; Novick et al., 1987, J. Biol. Chem. 262:8483-8487; Lomant and Fairbanks, 1976, J. Mol. Biol. 104:243-261; Hamada and Tsuruo, 1987, Anal. Biochem. 160:483-488; Hashida et al., 1984, J. Applied Biochem. 6:56-63.

Additionally, methods of cross-linking are reviewed by Means and Feeney, 1990, Bioconjugate Chem. 1:2-12.

Synthesis and characterization of targeting retro-inverted peptides

Similar to that described in the above-referenced WO 98/51325, synthetic dansylated peptides were manufactured at Genosys Biotechnologies, UK and Anaspec Inc., USA. Characterisation profiles included appearance, solubility, HPLC and mass spectrometry (minimum

purity >95%). Table 1 shows the primary sequences for both the retro-inverted peptide synthesized by reference to particular GIT targeting agents and the original GIT targeting agents themselves.

Table 1

Name	Description	Sequence
ZElan144	PAX2 15 mer fragment - D form retroinversion	K(dns)-rtrlrrnhsshkant
ZElan145	P31 16 mer fragment - D form retroinversion	K(dns)-gphrrgrpnsrsskrt
ZElan146	HAX42 14 mer fragment - D form retroinversion	K(dns)-gtsngngccnydgp
ZElan129	PAX2 15 mer fragment	K(dns)-TNAKHSSHNRRLRTR
ZElan031	P31 16 mer fragment	K(dns)-TRKSSRSNPRGRRHPG
ZElan091	HAX42 14 mer fragment	K(dns)-PGDYNCCGNGNSTG

Analysis of binding of dansylated peptides to Caco-2 cell membrane fractions by ELISA

Similar to the methods described in the above-referenced WO 98/51325, Caco-2 cell membrane (P100) and cytosolic (S100) fractions were prepared using a modification of the method described in Kinsella, B. T., O'Mahony, D. J. and G. A. FitzGerald, 1994, J. Biol. Chem. 269(47): 29914-29919. Confluent Caco-2 cell monolayers (grown in 75 cm^2 flasks for up to 1 week at 37°C and 5% CO_2) were washed twice in Dulbecco's PBS (DPBS) and the cells were harvested by centrifugation at 1000 rpm after treatment with 10 mM EDTA-DPBS. The cells were washed 3 times in DPBS and the final cell pellet was resuspended in 3 volumes of ice cold HED buffer (20 mM HEPES (pH 7.67), 1 mM EGTA, 0.5 mM dithiothreitol, 1 mM phenylmethylsulphonyl fluoride (PMSF)). The cells were allowed to swell for 5 min on ice prior to homogenization for 30 sec. The homogenates were

centrifuged at 40,000 rpm for 45 min at 4°C. The supernatant (S100) was removed and the pellet (P100) was resuspended in HEDG buffer (20 mM HEPES (pH 7.67), 1 mM EGTA, 0.5 mM dithiothreitol, 100 mM NaCl, 10% glycerol, 1 mM PMSF). Protein concentrations were determined using the Bradford assay (Bradford, M. M., 1976, Anal. Biochem. 72: 248-254).

Binding of peptides to membrane (P100) was assessed by detection of the dansyl moiety incorporated in the peptide. Costar ninety six well ELISA plates were coated with P100 fractions (100 μg/ml in 0.05 M $NaHCO_3$ (pH 9.6); 100 μl/well) overnight at 4°C. The plates were blocked with 2% Marvel-DPBS for 1 h at room temperature and washed 3 times in 1% DPBS-Tween. Peptides (200μg/ml in 2% Marvel-DPBS) were serially diluted on the plates and incubated for 1 h at room temperature. The plates were washed 5 times and the dansylated peptides were detected using i) mouse anti-dansyl antiserum (Cytogen DB3-226.3; 1:1340 dilution in 2% Marvel-DPBS) or ii) rabbit anti-dansyl antiserum (La Jolla Diagnostics LAJD-119; 1:1000 dilutions) for 1 h at room temperature. The plates were washed 3 times prior to incubation with i) goat anti-mouse IgGλ:HRP antibody (Southern Biotechnology 1060-05; 1:10,000 dilution in 2% Marvel-DPBS) or ii) anti-rabbit IgG HRP (Sigma A-0545, 1:8,000) for 1 h at room temperature. After 3 washes, reactons were visualized using K Blue Substrate and Red Stop Solution (Neogen Co. 300176 & 301475, respectively) at 650 nm.

ZElan021, full length HAX42 [K(dns)-SDHALGTNLRSDNAKEPG DYNCCGNGNSTGRKVFNRRRPSAIPT] was given the arbitrary value of 1.00 for binding to P100 at a given peptide concentration determined from the signal-to-noise ratio data. Table 2 shows the results of P100 assays with the HAX42 related peptides ZElan021, ZElan091 and ZElan146. Assay number 1 was at 20 μg/ml; 2 and 3 were at 50 μg/ml; and 4 through 8 were at 25 μg/ml. The results for the retro-inverted form, ZElan 146 show reasonable binding compared to the HAX42 fragment ZElan091 and that the activity of

the GIT targeting agent was not eliminated when converted to its retro-inverted form.

Table 2								
	P100 assay number							
Peptide	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
ZElan021	1.00	1.00	1.00	1.00	1.00	1.00	1.14	0.94
ZElan091				2.02	1.37	1.20	0.85	
ZElan146							0.36	0.72

K_D values, or the concentration of the peptide required to reach half maximal binding to Caco-2 P100 fractions, are given in Table 3 for ZElan021, full length HAX42, [K(dns)-SDHALGTNLRSDNAKEPGDYNCCGNGNSTGR KVFNRRRPSAIPT], HAX42 fragment ZElan091, and the retro-inverted form of this fragment, ZElan146 and well as for ZElan018, full length PAX2, [K(dns)-STPPSREAYSRPYSVDS DSDTNAKHSSHNRRLRTRSRPNG], PAX2 fragment ZElan129, and the retro-inverted form of this fragment, ZElan144.

Table 3		
Name	**Sequence**	**K_D (μmol)**
ZElan018	K(dns)-STPPSREAYSRPYSVDSDSDTNAKHSSHNRRLRTRSRPNG	>50.0
ZElan129	K(dns)-TNAKHSSHNRRLRTR	29.6
ZElan144	K(dns)-rtrlrrnhsshkant	28.8
ZElan021	K(dns)-SDHALGTNLRSDNAKEPGDYNCCGNGNSTGRKVFNRRRPSAIPT	6.7
ZElan091	K(dns)-PGDYNCCGNGNSTG	0.75
ZElan146	K(dns)-gtsngngccnydgp	21.65

Manufacture and analysis of peptide-coated insulin loaded PLGA particles

Insulin-loaded PLGA particles are coated with retro-inverted peptides according to this invention or GIT targeting agents by the coacervation processes described in the above-referenced WO 98/51325. In particular, solid particles containing an active agent are formed from a polymer and have a particle size of between about 10nm and 500 μm, most preferably 50 to 800 nm. In addition the particles contain targeting retro-inverted peptides which are incorporated into the particles using a number of methods as outlined below and described in the above-referenced WO 98/51325.

The organic phase (B) polymer of the general method given below may be soluble, permeable, impermeable, biodegradable or gastroretentive. The polymer may consist of a mixture of polymer or copolymers and may be a natural or synthetic polymer. Representative biodegradable polymers include without limitation polyglycolides; polylactides; poly(lactide-co-glycolides), including DL, L and D forms; copolyoxalates; polycaprolactone; polyesteramides; polyorthoesters; polyanhydrides; polyalkylcyanoacrylates; polyhydroxybutyrates; polyurethanes; albumin; casein; citosan derivatives; gelatin; acacia; celluloses; polysaccharides; alginic acid; polypeptides; and the like, copolymers thereof, mixtures thereof and stereoisomers thereof. Representative synthetic polymers include alkyl celluloses; hydroxalkyl celluloses; cellulose ethers; cellulose esters; nitrocelluloses; polymers of acrylic and methacrylic acids and esters thereof; dextrans; polyamides; polycarbonates; polyalkylenes; polyalkylene glycols; polyalkylene oxides; polyalkylene terephthalates; polyvinyl alcohols; polyvinyl ethers; polyvinyl esters; polyvinyl halides; poyvinylpyrrolidone; polysiloxanes and polyurethanes and co-polymers thereof.

Typically, particles are formed using the following general method:

An aqueous solution (A) of a polymer, surface active agent, surface stabilising or modifying agent or salt, or surfactant preferably a polyvinyl alcohol (PVA) or derivative with a % hydrolysis 50 - 100% and a molecular weight range 500 - 500,000, most preferably 80-100% hydrolysis and 10,000-150,000 molecular weight, is introduced into a vessel. The mixture (A) is stirred under low shear conditions at 10- 2000 rpm, preferably 100-600 rpm. The pH and/or ionic strength of this solution may be modified using salts, buffers or other modifying agents. The viscosity of this solution may be modified using polymers, salts, or other viscosity enhancing or modifying agents.

A polymer, preferably poly(lacide-co-glycolide), polylactide, polyglycolide or a combination thereof or in any enantiomeric form or a covalent conjugate of the these polymers with a targeting ligand is dissolved in water miscible organic solvents to form organic phase (B). Most preferably, a combination of acetone and ethanol is used in a range of ratios from 0:100 acetone: ethanol to 100: 0 acetone: ethanol depending upon the polymer used.

Additional polymer(s), peptide(s) sugars, salts, natural/biological polymers or other agents may also be added to the organic phase (B) to modify the physical and chemical properties of the resultant particle product.

A drug or bioactive substance may be introduced into either the aqueous phase (A) or the organic phase (B). A targeting retro-inversion peptide or GIT targeting agent may also be introduced into either the aqueous phase (A) or the organic phase (B) at this point.

The organic phase (B) is added into the stirred aqueous phase (A) at a continuous rate. The solvent is evaporated, preferably by a rise in temperature over ambient and/or the use of a vacuum pump. The particles are now present as a suspension (C). A targeting retro-inversion peptide or

GIT targeting agent may be introduced into the stirred suspension at this point.

A secondary layer of polymer(s), peptide(s) sugars, salts, natural/biological polymers or other agents may be deposited on to the pre-formed particulate core by any suitable method at this stage.

The particles (D) are then separated from the suspension (C) using standard colloidal separation techniques, preferably by centrifugation at high 'g' force, filtration, cross-flow filtration, gel permeation chromatography, affinity chromatography or charge separation techniques. The supernatant is discarded and the particles (D) re-suspended in a washing solution (E) preferably water, salt solution, buffer or organic solvent(s). The particles (D) are separated from the washing liquid in a similar manner as previously described and re-washed, commonly twice. A targeting ligand may be dissolved in washing solution (E) at the initial, intermediate and/or at the final washing stage and may be used to wash the particles (D).

The particles may then be dried. Particles may then be further processed for example, tabletted, encapsulated or spray dried.

The release profile of the particles formed above may be varied from immediate to controlled or delayed release dependent upon the formulation used and/or desired.

Drug loading may be in the range 0-90% w/w. Targeting retro-inversion peptide or GIT targeting agent loading may be in the range 0-90% w/w.

Insulin-loaded PLGA (RG504H) nanoparticles were manufactured as given above for the following targeting ligands: full length HAX42 (ZElan021), full length PAX2 (ZElan018), HAX42 fragment ZElan091, PAX2 fragment ZElan129, HAX42 fragment retro-inverted peptide ZElan146 and PAX2 fragment retro-inverted peptide ZElan144. Bovine insulin potency (HPLC)

and peptide loading (dansyl fluorescence) were assessed prior to analysis of insulin delivery in Wistar rats using the open loop model. Table 4 shows the insulin potency and targeting peptide loading of the PLGA particles.

Table 4

Peptide	Insulin Potency (mg/g)	Peptide Loading (μg/mg)
ZELAN018	47.0	1.68
ZELAN 129	59.0	0.85
ZELAN 144	58.2	0.68
ZELAN021	49.7	2.63
ZELAN 091	57.1	1.87
ZELAN 146	53.8	1.77

Animal Studies

In vivo assessment of oral insulin bioavailability of various targeting retro-inversion peptides was undertaken using open-loop studies study in which the test solution containing nanoparticles described above (Table 4) was injected directly into the ileum in Wistar rats similar to the protocols described in the above-referenced WO 98/51325. In short, Wistar rats (300-350g) were fasted for 4 hours and anaesthetized by intramuscular administration 15 to 20 minutes prior to administration of the test solution with a solution of ketamine [0.525 ml of ketamine (100 mg/ml) and 0.875 ml of acepromazine maleate-BP ACP (2mg/ml)]. The rats were then injected with a test solution (injection volume: 1.5ml PBS) intra-duodenally at 2-3 cm below the pyloris. Insulin (fast-acting bovine; 28.1 iu/mg) was incorporated in the particles as described above for a total of 300iu insulin (approximately 210 mg particles). Blood glucose values for the rats were measured using a Glucometer™ (Bayer; 0.1 to 33.3 m/mol/L); plasma insulin values were

measured using a Phadeseph RIA Kit™ (Upjohn Pharmacia; 3 to 240 μU/ml-assayed in duplicate). Systemic and portal blood was sampled.

Study groups included animals receiving test solutions containing particles coated with the following peptides shown in Table 4. Control groups included: 1) PBS control (1.5ml) Open-Loop; 2) Insulin solution (1iu/0.2ml) subcutaneous; 3) Insulin particles, no peptide.

Table 5 shows the insulin bioavailability for the insulin-loaded nanoparticles described above (surface modified with targeting retro-inversion peptide or GIT targeting agent) expressed as a % bioavailability of the administered oral dose compared to the reference insulin sub-cutaneous dose. Figures 1 and 2 show the (1) systemic blood glucose and (2) insulin levels following intestinal administration of control (PBS); ZElan 021 coated insulin-containing particles, ZElan 018 coated insulin-containing particles, ZElan091 coated insulin-containing particles, ZElan129 coated insulin-containing particles and ZElan 144 coated insulin-containing particles according to this invention (300iu insulin loading).

Table 5

Targeting Ligand	**% Insulin Bio-availability**
HAX42 ZELAN021	12.6
PAX2 ZELAN018	13.04
HAX42 14 MER ZELAN 091	11.4
HAX42 14 MER ZELAN 146 (retro-inversion)	2.1
PAX2 15 MER ZELAN 144 (retro-inversion)	10.8
PAX2 15 MER ZELAN129	14.3

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

CLAIMS

What is claimed is:

1. A retro-inverted peptide or a derivative thereof that specifically binds to a gastro-intestinal tract receptor selected from the group consisting of HPT1, hPEPT1, D2H, and hSI.

2. The retro-inverted peptide of claim 1 in which the peptide comprises an amino acid sequence selected from the group consisting of ZElan144, ZElan145 or ZElan 146 or a binding portion thereof.

3. A retro-inverted peptide that enhances delivery of an active agent across the gastro-intestinal tract into the systemic, portal or hepatic circulation.

4. The peptide of claim 1, wherein the peptide comprises no more than 50 amino acid residues.

5. The peptide of claim 1, wherein the peptide comprises no more than 40 amino acid residues.

6. The peptide of claim 1, wherein the peptide comprises no more than 30 amino acid residues.

7. The peptide of claim 1, wherein the peptide comprises no more than 20 amino acid residues.

8. A composition comprising the peptide of claim 1 bound to a material comprising an active agent, said active agent being of value in the treatment of a mammalian disease or disorder.

9. The composition of claim 8 in which the active agent is a drug.

10. The composition of claim 8 in which the material is a particle containing the active agent.

11. The composition of claim 8 in which the material is a slow-release device containing the drug.

12. The composition of claim 8 in which the peptide is covalently or noncovalently bound to the material.

13. A composition comprising a chimeric protein bound to a material comprising an active agent, in which the chimeric protein comprises a sequence selected from the group consisting ZElan144, ZElan145 or ZElan 146 or a binding portion thereof fused via a covalent bond to an amino acid sequence of a second protein, in which the active agent is of value in the treatment of a mammalian disease or disorder.

14. A composition comprising the peptide of claim 1 non-covalently bound to a particle containing a drug.

15. A composition comprising the peptide of claim 1 covalently bound to a drug.

16. The composition of claim 8 which facilitates the transport of the active agent through human or animal gastro-intestinal tissue.

17. The composition of claim 8 which targets the active agent to a selected site or selected tissue in a human or animal.

18. A method of delivering an active agent *in vivo* comprising administering to a subject a purified composition of claim 8.

19. A method of delivering a drug to a subject comprising administering to the subject a purified composition of claim 14.

20. A method of delivering a drug to a subject comprising administering to the subject a purified composition of claim 15.

21. The method according to claim 18 in which the administering is oral.

22. The method according to claim 18 in which the active agent is a drug.

23. The method according to claim 18 in which the subject is a human.

24. The method according to claim 21 in which the subject is a human.

25. The method according to claim 18 in which said composition facilitates the transport of the active agent through human or animal gastro-intestinal tissue.

26. The method according to claim 19 in which the administering is oral.

27. A pharmaceutical composition comprising the composition of claim 8 in a pharmaceutically acceptable carrier suitable for use in humans *in vivo*.

28. An antibody which is capable of immunospecifically binding the peptide of claim 1.

29. A molecule comprising a fragment of the antibody of claim 28, which fragment is capable of immunospecifically binding said peptide.

30. A purified derivative of the peptide of claim 1, which displays one or more functional activities of said peptide.

31. The derivative of claim 30 which is able to be bound by an antibody directed against said peptide.

32. A fragment of the peptide of claim 2 comprising a domain of said peptide.

33. A fragment of the peptide of claim 3 comprising a domain of said peptide.

34. A pharmaceutical composition comprising a therapeutically effective amount of a composition comprising the peptide of claim 1 and a pharmaceutically acceptable carrier.

35. A method of treating or preventing a disease or disorder comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of the composition of claim 8.

36. A method of treating or preventing a disease or disorder comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of the composition of claim 14.

37. A method of treating or preventing a disease or disorder comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of the composition of claim 15.

38. The method according to claim 35 in which the disease or disorder is selected from the group consisting of: hypertension, diabetes, osteoporosis, hemophilia, anemia, cancer, migraines, and angina pectoris.

39. The method according to claim 38 in which the subject is a human.

40. A composition comprising the peptide of claim 1, wherein the peptide is coated onto or absorbed onto or covalently bonded to the surface of a nano- or microparticle.

41. A nano- or microparticle formed from the peptide of claim 1.

42. The composition of claim 40, wherein the nano- or microparticle is a drug-loaded or drug-encapsulating nano- or microparticle.

43. The composition of claim 8 in which the drug is insulin or leuprolide.

ABSTRACT OF THE DISCLOSURE

Retro-inverted forms of GIT targeting agents that target specific receptor sites *in vivo* and/or promote uptake of active agents and/or enhance active agent delivery across the GIT into the systemic circulation are provided. These retro-inverted peptides and compositions containing these retro-inverted peptides can be used to deliver an active agent, such as a drug or a drug-containing nano- or microparticle for treatment of a condition in a subject in need of the drug, *in vivo*. Additionally, the invention provides antibodies which are capable of immunospecifically binding the retro-inverted peptides.

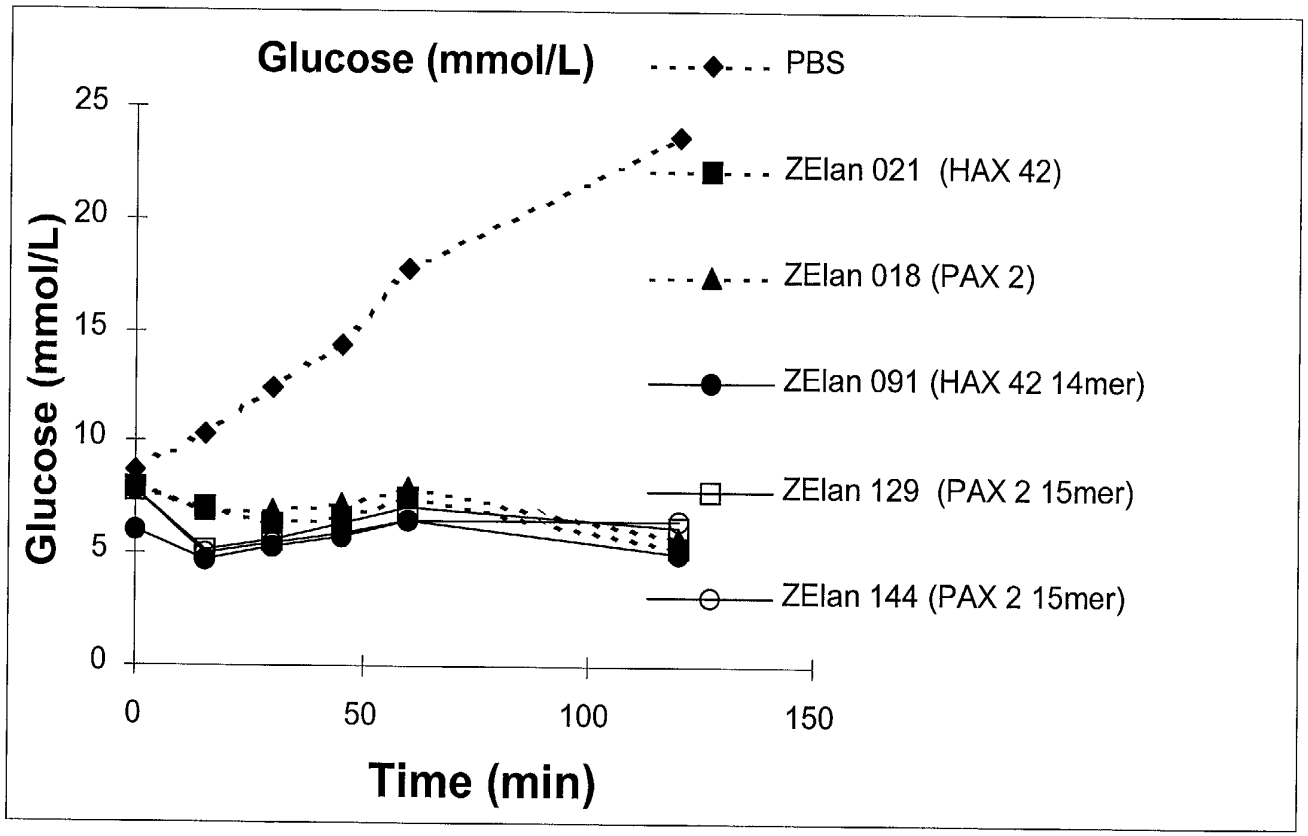

Fig. 1

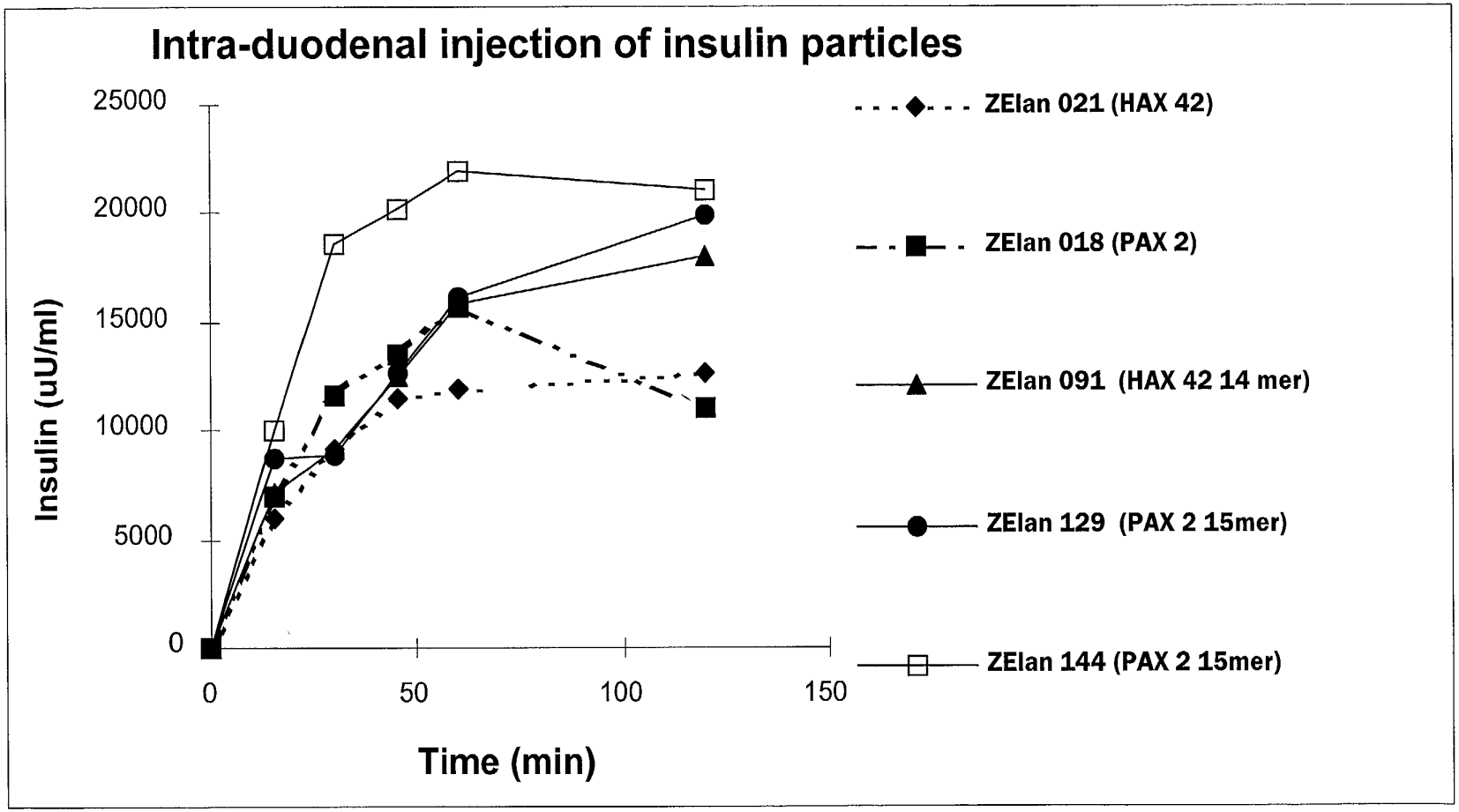

Fig. 2

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

(X) Original ( ) Supplemental ( ) Substitute ( ) PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"Retro-inversion peptides that target GIT receptors and related methods"**, the specification of which:

(check one)
[X] is attached hereto, or
[ ] was filed on _____, as Application Serial No. _____ and with amendments through _____ (if applicable), or
[ ] Express Mail No., *as Serial No. not yet known* _____,
[ ] was described and claimed in PCT International Application No. _____, filed _____, and as amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me or relating to this subject matter having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE
60/109,038	November 19, 1998

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT International application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

APP. SER. NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED

I hereby appoint the following attorneys and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full name of second inventor: _____.
Inventor's signature: _____. Date: _____
Residence: _____.
Citizenship: _____. Post Office Address: _____.

☐ Additional inventors are being named on separately numbered sheets attached hereto